THE INHIBITORY ACTION OF CERTAIN ANILIN DYES UPON BACTERIAL DEVELOPMENT.

By Charles E. Simon, B.A., M.D.,

PROFESSOR OF CLINICAL PATHOLOGY AND EXPERIMENTAL MEDICINE AT THE COLLEGE OF PHYSICIANS AND SURGEONS, BALTIMORE, MARYLAND,

ASSISTED BY

Martha A. Wood, M.D.,

OF HOUSTON, TEXAS.

SECOND COMMUNICATION.[1]

In a recent communication[2] we showed that the inhibitory action which certain violet dyes, like gentian violet, methyl violet, Hoffmann's violet, and others, exercise upon certain bacteria is intimately dependent upon the triphenyl methane structure of these dyes and upon the presence of the three amino groups which are found in all the inhibitory representatives of this class. We have also pointed out that the replacement of the basic by acid auxochromic groups or a diminution of the basicity of the respective compounds by the additional introduction of acid groups leads to a loss of this inhibitory power.

In view of the practical as well as theoretical aspects of this problem, it seemed of interest to us to extend our studies to other groups of dyes also, and it is the purpose of this second paper to present the results of these investigations.

Dyes Investigated. Our studies include representatives of the diphenyl amines (that is, of the indamins and indophenols, the oxazins and oxazons, the thiazins and thiazons, and the azins) of the acridins, of the azo dyes, of the hydrazons, of the azobenzidins, of the thiazols, and the oxyquinones.

The general technique was the same as that described in our first communication, the various dyes being used in the concentration of 1 in 100,000 of nutrient agar. The list includes the following representatives, and has been arranged in accordance with their structural composition and their basicity or acidity, the presence of acid or basic groups in addition to the predominating types being represented by arrows pointing in the direction of acidity or basicity respectively.

The organisms examined were for the most part the same as those which had been used in our investigations of the triphenyl methanes.

Diphenyl Amines.

A. Indamins and Indophenols.

Basic.	Acid.
Phenylene blue	
Bindschedler's green	

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[1] Received for publication May, 1913.

[2] Amer. Jour. Med. Sci., vol. cxlvii, No. 2, p. 217.

B. Aurantia.

	Aurantia

C. Oxazins and Oxazons.

Capri blue	←——Gallocyanin
Muscarin——→	
Nile blue	
New methylene blue	
Coelestin blue——→	

D. Thiazins and Thiazons.

Thionin	←——Thiocarmin
Methylene blue	
Ethylene blue	
Toluidin blue	
Methylene green——→	
Methylene violet	
Methylene azure	

E. Azins.

Eurhodins and eurhodoles:	Indulin R.
Neutral red	Indulin B.
Neutral violet	
Safranins and safranoles:	
Phenosafranin	
Tolusafranin	
Tannin heliotrope	
Amethyst violet	
Echt neutral violet	
Paraphenylene blue	
Indazin	
Indulins and indones:	
Naphtazin blue	Naphtazin blue (sulphonic acid derivative)
Aposafranins:	
Neutral blue	Rosindulin G.
Basel blue	

Azo Dyes.

Anilin yellow	←——Methyl orange
Chrysoidin	Orange G.
Vesuvin	Ponceau
Indolin blue	Roccellin
	Alizarin yellow
	←——Citronin
	Dianil red
	Dianil blue
	←——Dianil black
	Tartrazin

Azobenzidins.

	←——Benzopurpurin
	←——Diamin violet
	←——Diamin blue
	←——Diamin green
	←——Pyramin orange
	←——Congo red

Thiazols.

Primulin	

Quinolins and Acridins.

Acridin orange	←——Acid quinolin yellow

Oxyquinones.

	Alizarin red W. S.
	Acid alizarin blue

The results which have been obtained with these various dyes in the case of the different organisms that have been examined will be found grouped together in the accompanying tables, the acid dyes being italicized.

TRIPHENYL

Triamino-triphenyl methanes.

		Fuchsin.	New fuchsin.	*Acid fuchsin.*	Methyl violet.	Crystal violet.	Hofmann's violet.	Benzyl violet.	Dahlia.	*Roth violet 5RS.*	Iodine green.	Methyl green.	Ethyl green.	*Acid violet.*	*Echt green.*
St. aureus	1	0	0	+++	0	0	0	0	0	+++	+++	± to +	+	+++	+++
	96	+++	0 to ++	+++	–	0	0	0 to +	±	–	+++	++	++	+++	+++
	40	+++	0	+++	0	0	0	0	±	+++	+++	+++	+	+++	+++
	95	+++	+++	+++	0	0	0	0 to ±	0	+++	+++	+++	++	+++	+++
	42	0	0	+++	0	0	0	0	0	+++	+++	+++	+++	+++	+++
	83	+++	+++	+++	0	0	0	±	0	+++	+++	+++	+	+++	+++
	53	+++	+++	+++	0	+	0	0	0	+++	+++	+++	++	+++	+++
	84	+++	0	+++	0	±	0	0	0	+++	+++	+++	0	+++	+++
	58	–	–	–	0	0	0	–	0	+++	+++	∓ to +	–	+++	+++
St. pyogenes	2	0	–	–	0	–	–	–	0	–	–	0 to ∓	–	–	–
	92	+++	+	+++	0	++	++	0	+++	+++	+++	+++	0	+++	+++
	26	0	–	–	0	0	–	–	0	–	–	+	–	–	–
	93	–	–	+++	+	–	–	–	–	+++	–	–	–	–	–
	28	0	–	–	±	0	–	–	0	–	–	–	–	–	–
	82	–	+++	+++	–	–	–	0	–	+++	–	++	0	±	–
	43	0	–	–	0	0	–	–	0	–	–	0	–	–	–
	85	0	0	+++	0	0	0	0	0	+++	+++	0	0	+++	+++
	50	0	0	+++	0	0	0	0	0	+++	+++	+++	0	+++	+++
	81	+	+++	+++	0	0	–	0	+++	+++	+++	0	0	+++	+++
	54	+++	0	+++	0	++	+	0	+++	+++	+++	+++	0	+++	+++
	57	0	–	–	0	–	0	–	0	+++	++	++	–	–	–
	77	+++	+++	+++	+++	0	+++	0	+++	+++	+++	+++	0	–	+++
	61	0	–	–	0	0	0	–	0	+++	+++	0	–	+++	+++
	78	–	..	–	0	0	+++	–	0	+++	..	0	–	+++	+++
	16	+++	+++	+++	+++	+++	–	+++	+++	+++	+++	+++	+++	+++	+++
	37	0	0	+++	0	0	0	0	0	+++	+++	+	0	+++	+++
	47	–	–	–	0	–	–	..	0	+++	–	0	–	–	–
St. citreus	44	0	0	+++	0	0	0	∓ to +	0	+++	+	0	+	+++	+++
M. catarrhalis	6	0	0	+++	0	0	0	0	0	+++	+	∓ to +	+	+++	+++
	100	+++	0	+++	–	–	0	∓	0	+++	+++	+++	0	+++	+++
	41	0	–	–	0	0	0	–	0	+++	+++	0	–	+++	+++
	75	–	–	–	0	0	–	–	–	–	–	–	–	–	–
M. aurantiacus	9	0	0	+++	0	++	0	±	0	+++	+++	++	+	+++	+++
Anthrax	14	0 to ±	0	+++	0	0	0	0	0	+++	+++	+++	0	+++	+++
Actinomyces	15	0 to ±	0	+++	0	0	0	0	0	+++	+++	++	0	+++	+++
Pneumococcus	102	0	–	+++	0	0	–	–	0	–	–	–	0	++	–
	64	±	–	–	+	+	0	–	±	–	+++	+++	–	+++	+++
	29	±	–	–	0	0	–	–	0	–	–	+	–	–	–
Meningococcus	71	–	–	–	0	0	–	–	–	+++	+++	++	–	–	+++
	105	+++	–	–	+++	+++	+++	+++	+++	–	–	–	–	–	–
B. typhosus	3	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
B. paratyphosus	11	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
B. paratyphosus	12	+++	+++	+++	+++	+++	++	+++	+++	++	+++	+++	+++	+++	+++
B. paracoli	19	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
B. coli	32	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
B. enteritidis	8	+++	+++	+++	0	0	0	–	0	+++	+++	+++	+++	+++	+++
B. dysenteriæ	67	+++	+++	+++	+++	++	+++	+++	++	+++	+++	+++	++	+++	+++
	90	+++	+++	+++	+++	+++	+++	+++	+++	–	+++	+++	++	+++	+++
	68	+++	+++	+++	+++	++	+++	+++	++	+	+++	+++	++	++	+++
	69	+++	+++	+++	+++	++	+++	+++	+++	+++	+++	+++	+	+++	+++
B. pyocyaneus	70	++	+++	+++	+++	++	+++	+++	++	+	++	+++	++	+++	+++
	7	+++	–	–	+++	+++	+++	–	+++	+++	+++	+++	–	++	+++
B. subtilis	4	0	–	–	0	0	0	–	0	+++	+++	0	–	+++	+++
	13	+++	–	–	0	0	–	0	0	+++	+++	+++	–	+++	–
V. choleræ	20	+++	..	..	+++	..	..	+++	..	..	..	+++			

METHANES.

DIPHENYL METHANES.

Oxytriphenyl methanes.		Phthaleins.				Pyronins.						Diaminotriphenyl methanes.				
Aurin.	*Corallin.*	*Fluorescein.*	*Uranin.*	*Eosin.*	*Erythrosin.*	Rhodamin. 6 G.	Rhodamin. 3 B	Rosazein, 6 G.	Rosazein, B.	*Sulphorosazein, B.*	Pyronin.	Malachite green, FFO.	Brilliant green.	*Acid green.*	Auramin. G.	Auramin. OO.
+++	+++	+++	+++	+++	+++	0	0	+++	0	+++	+++	+	+++	+++	+++	+++
+++	+++	+++	+++	+++	+++	+	∓	+++	0 to ±	+++	+++	+	+	+++	+++	+++
+++	+++	+++	+++	+++	+++	−	0	+++	0	+++	+++	+++	+	+++	+++	+++
+++	+++	+++	+++	+++	+++	0	∓	+++	0	+++	+++	±	+	+++	+++	+++
+++	+++	+++	+++	+++	+++	0	+	+++	0	+++	+++	+++	0 to ∓	+++	+++	+++
+++	+++	+++	+++	+++	+++	+	±	+++	0	+++	+++	+++	0 to ±	+++	+++	+++
+++	+++	+++	+++	+++	+++	0	±	+++	0	+++	+++	+	+++	+++	+++	+++
+++	+++	+++	+++	+++	+++	0	±	+++	0	+++	+++	+	0 to ±	+++	+++	+++
+++	−	−	−	−	−	−	−	−	−	−	−	−	+++	−	−	−
−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
+++	++	+++	++	+++	−	+++	++	+++	+ to ++	+++	+++	0	+	+++	+++	+++
−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	−	−	+++	−	+++	−	−	−	−	−	−	−	−	+++	−
−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−	+++	−	+++	−	+++	−	−
−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
+++	++	+++	+++	+++	+++	+++	+++	+++	++	−	−	++	0	−	+++	+++
+++	+++	+++	+	+++	+++	0	−	+++	0	+++	+++	0	0	−	+++	+++
+++	+++	+++	+++	+++	+++	−	++	+++	+ to ++	+++	+++	++	0	+++	+++	+++
+++	+	+++	+++	+++	+++	+++	+++	+++	+++	−	+++	0	0	−	+++	+++
−	−	−	−	−	−	−	−	−	−	−	−	−	0	−	−	−
+	+++	+++	+++	+++	+++	++	+++	+++	0	−	+++	0	0	−	+++	+++
−	+++	−	+++	−	−	−	−	−	−	−	−	−	0	−	−	−
−	−	−	+++	−	−	−	−	−	−	−	−	−	0	−	−	−
+++	+++	−	++	+++	+++	+++	+++	+++	0	−	+++	+++	+++	−	+++	+++
+++	+++	+++	+++	+++	+++	+++	+++	+++	0	+++	+++	0	0	−	+++	+++
−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
+++	+++	+++	+++	+++	+++	∓	+	+++	0	+++	+++	0 to +	+++	+++	+++	+++
+++																
+++	+++	+++	+++	+++	+++	0	0	+++	0	+++	+++	+++	++	+++	+++	+++
+++	+++	++	+	++	+++	0	0	+++	0	+++	+++	+	0 to ±	+++	+++	+++
−	+++	−	+++	++	−	+	0	−	−	−	−	−	+++	−	−	−
−	−	−	+++	−	−	−	−	−	−	−	−	−	−	−	−	−
+++	+++	+++	+++	+	++	0	0	+++	0	+++	+++	0 to +	+++	+++	+++	+++
+++	+++	+++	+++	+++	+++	0	0	+	0	+++	+++	0	+++	+++	+++	+++
+++	+++	+++	+++	+++	+++	0	0	+	0	−	+++	0 to ±	+	+++	+++	+++
−	++	+++	+	−	+++	−	−	−	+++	−	−	++	0	+++	−	−
−	++	+++	−	−	−	−	−	−	−	−	−	−	0	−	−	−
−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−	−	−	−	0	−	−	−
−	−	−	−	−	−	−	−	−	−	−	−	−	+++	−	−	−
+++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+++	+++	+++
+++	+	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	0 to ±	+	+++	+++	+++
+++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+	++	+++	+++	+++
+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+++	+++	+++	+++
+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+++	+++	+++	+++
+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+	+++	+++	+++	+++
+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+	+++	+++	+++	+++
+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+	+++	+++	+++	+++
+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+	+++	+++	+++	+++
+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+++	+++	+++	+++
−	+++	+++	+++	+++	−	+++	+++	−	−	−	+++	−	−	−	+++	−
−	+++	+++	+++	+++	−	0	0	−	−	−	+	−	−	−	+	++
−	+++	+++	−	−	−	+++	0	−	−	−	+++	−	−	−	++	+

QUINOLINS AND ACRIDINS.

				Indamins.		Aurantia.	Oxazins and Oxazons.								
		Quinolin yellow.	Acridin orange.	Phenylene blue.	Bindschedler's green.	*Aurantia.*	Capri blue.	Muscarin.	Nile blue.	New methylene blue, GGN.	*Gallocyanin.*	Coelestin blue.	Ultracyanin.	Thionin.	Methylene blue.
St. aureus	1	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	40	+++	+++	..	..	+++	++	+++	+++	0 to ±	+++	+++	+++	+++	+++
	96	+++	+++	+++	++	+++	+++	+++	+++	+	+++	+++	+++	+++	++
	42	+++	+++	..	..	+++	± to +	+++	+++	+++	+++	+++	+++	+++	+++
	95	+++	+++	..	..	+++	+++	+++	+++	0	+++	+++	+++	+++	++
	53	+++	+++	..	..	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	83	+++	+++	..	..	+++	+	+++	+++	+++	+++	+++	+++	+++	++
	84	+++	+++	..	..	+++	++	+++	+++	0	+++	+++	+++	+++	+
St. pyogenes	82	+++	+++	..	..	+++	–	–	–	2 0 26 0	–	0	–	–	–
	92	+++	+++	..	..	+++	++	+	++	92 0	+++	0	+++	+++	++
	93	–	–	..	..	++	+++	–	+	–	–	–	–	–	–
	50	+++	+++	+++	0	+++	0	+++	+	+++	+++	0	+++	+++	++
	85	+++	+++	..	..	+++	0	–	0	0	+++	0	+++	+++	0
	54	+++	+++	..	..	+++	0	+++	+++	0	+++	0	+++	0	0
	81	+++	+++	..	..	+++	0	+++	0	+	+++	0	+++	+++	0
	57	–	–	..	..	–	–	+++	+	0	–	..	–	0	–
	77	–	+++	..	..	+++	0	–	+++	–	+++	0	+++	+++	–
	61	–	–	..	..	–	–	+++	0 to ∓	0	+++	–	–	–	0
	78	–	–	..	..	–	+++	–	++	–	+++	–	+++	–	–
	10	+++	+++	+++	0	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	32	+++	+++	+++	0	+++	+++	+++	+	+++	+++	0	+++	+++	+++
St. citreus	44	+++	+++	..	..	+++	++	+++	++	0	+++	+++	+	+++	0
M. aurantiacus	9	+++	+++	..	..	+++	+++	++	+	+	+++	+++	+++	+++	+++
M. catarrhalis	6	+++	+++	..	..	+++	+	+++	++	0	+++	+++	+++	+++	+++
	100	+++	+++	+++	0	+++	±	+++	++	0	+++	+	+++	+++	++
	76	–	–	..	..	++	0	–	–	–	–	–	+++	–	–
	41	–	–	..	..	++	0	+++	0	0	+++	–	++	–	–
Pneumococcus	102	–	–	..	..	–	–	+	–	–	–	0	–	–	+
	64	–	–	..	..	–	–	+++	+++	0	+	–	–	–	–
Meningococcus	71	–	–	..	..	–	–	+++	+	–	+++	–	–	–	–
	105	–	–	..	..	..	..	..	..	+++	..	..	..	..	±
Anthrax	14	+++	+++	+++	+	+++	± to + 0 to +++	+++	+++	++	+++	0 to ∓	+++	0 to ++	0 to +
Actinomyces	15	+++	+++	..	..	+++	0 to +++	+++	+++	++	+++	0 to ±	+++	0 to ±	0 to ++
B. typhosus	3	+++	+++	..	..	+++	+++	+++	+	+++	+++	+++	+++	+++	++
—α B. paratyphosus	11	+++	+++	..	..	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
—β	12	+++	+++	..	..	+++	+++	+++	+++	+++	+++	+++	+++	0	+++
B. paracoli	19	+++	+++	..	..	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
B. coli	32	+++	+++	..	..	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
B. dysenteriæ	67	+++	+++	..	..	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	90	+++	+++	..	..	+++	+++	+++	+++	–	–	+++	+++	+++	+++
	68	+++	+++	..	..	+++	+++	+++	+++	+++	+	+++	+++	+++	+++
	69	+++	+++	..	..	+++	+++	+++	+++	++	+++	+++	+++	+++	+++
	70	+++	+++	..	..	+++	+++	+++	+++	0	++	+++	+++	+++	+++
B. enteritidis	8	+++	+++	+++	+++	+++	+++	+++	0	+	+++	+++	+++	+++	+++
B. subtilis	4	–	–	..	..	+++	–	–	–	–	–	–	–	–	±
	13	–	–	..	..	+++	–	–	–	–	–	–	–	–	–
B. pyocyaneus	7	–	–	..	..	+++	–	–	–	–	–	–	–	–	–

DIPHENYLAMINS.

Thiazins and Thiazons.							Eu-rho-dins.	Indulins and Indones.						
Ethylene blue.	Toluidin blue.	Methylene green.	*Thiocarmin, R.*	Methylene violet.	Methylene azure.	Uranin blue.	Neutral red.	Naphtazin blue conc.	Naphtazin blue 3157	Indazin, M.	Paraphenylene blue.	Azin red 5 RN.	*Indulin, R.*	*Indulin, B.*
+++	+++	+++	+++	± to +	+++	+++	+++	+++	+	0	+++	+++	+++	+++
+++	+++	+++	+++	+++	+++	+++	+++	+++	± to ++	+++	..	± to +	+++	+++
+++	+++	+++	+++	++	+++	+++	+++	+++	+	0 to ±	+++	+++	+++	+++
+++	+++	+++	+++	+	+++	+++	+++	+++	+++	0	..	+++	+++	+++
+++	+++	+++	+++	—	+++	+++	+++	+++	+	0 to ±	..	+++	+++	+++
+++	+++	+++	+++	—	+++	+++	+++	+++	+++	0	..	+++	+++	+++
+++	+++	+++	+++	++	+++	+++	+++	+++	+	+	..	+++	+++	+++
+++	+++	+++	+++	++	+++	+++	+++	+++	+	0 to +	..	+++	+++	+++
—	—	—	—	—	—	—	—	—	—	—	..	—	—	—
0	+++	+++	+++	—	++	+++	+++	+++	++	0	..	++	+++	+++
—	+++	—	—	+++	0	+	+++	+++	+++	+	..	++	+++	+++
0	+++	+++	++	+++	+++	+++	+++	+++	0	0	+++	0	+++	+++
0	+++	+++	+++	+++	+++	+++	+++	+++	0 to +	0	..	0	++	+++
+++	+++	+++	++	+++	+	+++	+++	+++	+++	0	..	0	+++	+++
0	++	+++	± to +	+++	+	+++	+++	+++	++	0	..	0	++	+++
—	—	—	—	—	—	—	—	—	—	0	..	0	—	—
0	+++	+++	+++	++	+	+++	+++	+++	++	0	..	0	+++	+++
—	—	—	+++	—	—	—	—	+++	—	0	..	0	—	—
—	—	—	—	—	—	+++	—	+++	0	0	..	0	+++	+++
+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+ to ++	+++	+++	+++	—
0	+++	+++	+++	+++	+++	+++	+++	+++	+++	0	+++	0	+++	+++
0	+++	+++	+++	—	++	+++	+++	+++	+	0	..	0	+++	+++
+	+++	+++	++	0	+	+++	+++	+++	+++	0	..	+	+++	+++
+	+++	+++	+++	0	+++	+++	+++	+++	0 to ±	0	..	+++	+++	+++
0 to ∓	++	+	+++	++	0	+++	+++	+++	+	—	+++	±	+++	+++
—	—	—	—	—	—	—	—	+++	+	+++	..	0	+++	+++
—	—	—	—	—	—	—	—	+++	0	0	..	0	+++	+++
0	+++	—	++	—	0	+++	—	+++	++	—	..	±	+++	—
—	—	—	—	—	—	—	—	+++	++	—	..	—	—	—
—	—	—	—	—	—	—	—	+++	0 to ±	—	..	—	+++	+++
+++	+++	+++	+++	±	0	+ to ++	+++	+++	+	0	+++	0	+++	+++
..	..	..	..	..	..	..	..	..	+					
+++	+++	+	+++	0	++	+++	+++	+++	+++	0	..	0	+++	+++
+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	..	+++	+++	+++
+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	..	+++	+++	+++
0	++	+++	+++	++	+++	+++	+++	+++	+++	+++	..	+++	+++	+++
+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	..	+++	+++	+++
+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	..	+++	+++	+++
+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	..	+++	+++	+++
+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	..	+++	+++	+++
+++	+++	+++	+++	+++	0 to +	+++	+++	+++	+++	+++	..	+++	+++	+++
+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	..	+++	+++	+++
+++	+++	+++	+++	+++	0 to +	+++	+++	+++	+++	+++	..	+++	+++	+++
+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	..	+++	+++	+++
—	—	—	—	—	—	+++	—	+++	—	—	..	+	—	—
—	—	—	+	—	—	—	—	—	—	—	..	0	—	—
—	—	—	—	—	—	+++	—	—	—	+++	..	—	—	—

DIPHENYLAMINS (Continued).

		Safranins and Safranoles.					Aposafranins.				Simple azo anilins and Naph-				
		Phenosafranin.	Tolusafranin.	Tannin-heliotrope.	Amethyst.	Echt neutral violet.	Neutral blue.	Basel blue, R. conc.	*Rosindulin, 2 B.*	*Rosindolin, 2 G.*	Anilin yellow.	*Methyl orange.*	Chrysoidin.	Vesuvin.	*Orange, G.*
St. aureus	1	+++	+++	+++	+++	+++	+++	++	+++	+++	+++	+++	+++	+++	+++
	40	+++	+++	+++	+++	± to +	..	++	+++	+++	..	+++	+++	+++	+++
	42	+++	+++	+++	+++	0 to ±	..	+	+++	+++	+++	+++	+++	+++	+++
	95	±	+++	++	+++	0	..	+++	+++	+++	..	+++	+++	+++	+++
	96	±	++	+	+++	0 to ±	+	+++	+++	+++	..	+++	+++	+++	+++
	53	+++	+++	+	+++	+++	..	+	+++	+++	+++	+++	+++	+++	+++
	84	+++	+++	+++	+++	+	..	+++	+++	+++	..	+++	+++	+++	+++
	58	+++	+++	±	–	0	..	+	+++	+++	+++	–	–	–	–
	83	+++	+++	+++	+++	0 to +	..	+++	+++	+++	..	+++	+++	+++	+++
St. pyogenes	93	–	–	+++	0	+++	..	+++	+++	+++	..	–	+++	–	–
	54	+++	0	0	+++	++	+++	+++	+++	+++	..	+++	+++	+++	+++
	85	+++	++	0	+++	+++	..	+++	+++	+++	..	+++	+++	+++	+++
	92	0	+++	0	+++	+++	..	+++	+++	+++	..	+++	+++	+++	+++
	50	0	–	0	+++	+	+++	+++	+++	+++	+++	+++	+++	+++	+++
	81	± to +	+++	0	+++	+++	..	+	+++	+++	..	+++	+++	+++	+++
	7'	0	0	0	++	+++	..	+++	+++	+++	+++	+++	+++	+++	+++
	78	0	0	0	+++	+++	..	–	+++	+++	..	–	–	–	–
	37	+++	+++	+++	+++	+++	+++	+++	+++	+++	..	+++	+++	+++	+++
	10	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
St. citreus	44	+	+	0 to ±	++	0 to ±	..	0 to ±	+++	+++	+++	+++	+++	–	+++
M. aurantiacus	9	0 to ±	–	0	+++	0	..	+	+++	+++	+++	+++	+++	+++	+++
M. catarrhalis	6	+++	+++	0	+++	0	..	0 to +	+++	+++	..	+++	+++	+++	+++
	75	+++	+++	+	0	++	..	–	+++	–	+++	–	+++	–	+++
	41	..	..	0	–	–	..	0	+++	–	..	+++	–	–	–
	100	++	++	±	+++	0	+	0	+++	+++	+++	+++	–	+++	+++
Pneumococcus	101	+++	–	–	–	+	..	–	+++	+++	–	–	–	–	–
	102	–	–	–	–	–	..	–	–	–	+++	–	+++	+++	+++
Meningococcus	71	+++	–	–	++	–	..	–	++	+++	–	–	–	–	–
Anthrax	14	+++	+++	0 to ±	+++	0	+++	+ to ++	+++	+++	+++	+++	+++	+++	+++
Actinomyces	15	++	+	0 to ±	++	0 to +	..	..	+++	+++	+++	+++	+++	+++	+++
B. typhosus	3	+++	+++	+++	+++	+++	..	+++	+++	+++	+++	+++	+++	+++	+++
—α B. paratyphosus	11	+++	+++	+++	+++	+++	..	+++	+++	+++	+++	+++	+++	+++	+++
—β	12	+++	+++	+++	+++	–	..	+++	+++	+++	+++	+++	+++	+++	+++
B. paracoli	19	+++	+++	+++	+++	++	..	+++	+++	+++	..	+++	+++	+++	+++
B. coli	32	+++	+++	+++	+++	+++	..	+++	+++	+++	..	+++	+++	+++	+++
B. enteritidis	8	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
B. dysenteriæ	67	+	+++	+++	+++	+++	..	+++	+++	+++	+	+++	+++	+++	+++
	68	+	+++	+++	+++	+++	..	+++	+++	+++	++	+++	+++	+++	+++
	69	+++	+++	+++	+++	+++	..	+++	+++	+++	++	+++	+++	+++	+++
	70	+++	+++	+++	+++	++	..	+++	+++	+++	+	+++	+++	+++	+++
	90	+++	–	–	+++	+++	..	+++	+++	+++	–	+++	+++	+++	+++
	91	–	–	–	–	+++	..	+++	+++	+++	–	–	–	–	–
B. subtilis	4	–	–	–	–	–	..	0	–	+++	–	–	–	–	–
	13	–	–	–	–	–	..	–	–	+++	–	–	+++	–	–
B. pyocyaneus	7	–	–	–	–	–	..	–	–	..	–	–	–	–	–

AZO DYES.

thylamins.		Chromotropes.			Azodiphenylamins.	Diazo bodies.		Hydrazones.	Azo benzidins.						Oxyquinones.	
Ponceau.	*Roccellin.*	*Dianil red.*	*Dianil blue.*	*Dianil black.*	*Citronin, 000.*	Indoin blue.	Diazin blue, BR.	*Tartrazin.*	*Pyramin orange.*	*Congo red.*	*Benzopurpurin.*	*Dianin.*			*Alizarin blue.*	
												Green.	*Blue.*	*Violet.*	*W.S.*	*BB.*
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+++	+++	+++	+++	+++	+++	++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++
+++	+++	+++	+++	+++	+++	++	± to +	+++	+++	+++	+++	+++	+++	+++	+++	+++
+++	+++	+++	+++	+++	+++	+++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++
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+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	–	+++	+++	–	+++	+++
+++	+++	+++	++	+++	+++	+++	+++	+++	+++	+++	–	+++	–	+++	+++	+++
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+++	+++	+++	+++	+++	+++	+++	+	+++	+++	+++	–	+++	+++	–	+++	+++
+++	+++	+++	+++	+++	+++	++	+	+++	+++	+++	+++	+++	+++	+++	+++	+++
+++	+++	–	–	–	+++	++	++	+++	–	+++	+++	+++	+++	+++	+++	+++
–	–	–	–	–	–	+++	+++	+++	–	–	–	–	–	–	+++	+++
+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
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+++	+++	+++	+++	+++	+++	+++	+	+++	+++	+++	+++	+++	+++	+++	+++	+++
+++	+++	+++	+++	+++	+++	0 to +	0 to +	+++	+++	+++	–	+++	+++	+++	+++	+++
+++	+++	+++	+++	+++	+++	+++	0	+++	+++	+++	–	+++	+++	+++	+++	+++
+++	–	–	–	–	–	0	+++	+++	–	–	–	–	–	–	+++	+++
–	–	–	–	–	–	++	0	+++	–	+++	–	–	–	–	–	–
+++	+++	–	–	–	+++	±	0 to ±	+++	–	–	–	+++	+++	+++	+++	+++
–	–	–	–	–	+++	–	–	+++	–	–	–	–	–	–	+++	+++
–	–	+++	+++	–	–	+++	–	+++	+++	–	+++	+++	+++	+++	+++	+++
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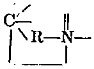
–	–	–	–	–	+++	+	–	+++	+++	+++	+++	–	–	+++	–	+++
–	–	–	–	–	–	+++	–	+++	–	+++	+++	–	–	–	–	–
–	–	–	–	–	–	–	–	+++	–	–	+++	–	–	+++	–	–

BEHAVIOR OF THE ACID DYES. A survey of these tables will show that not a single one of the acid dyes proper possesses any inhibitory properties in the standard concentration of 1 to 100,000 which we employed. In a number of instances, indeed, we received the impression as though a favoring effect were exercised upon the growth of those organisms, which ordinarily are inhibited by the triamino-triphenyl methanes. This seemed notably the case with tartrazin, a strongly acid hydrazone; then with Ponceau, an azo dye; further with gallocyanin, an oxazon, and the same had previously been noted in one of the triphenyl methanes, that is, roth violet 5 RS. So manifest was this favoring influence in the case of streptococci that the thought suggested itself that tartrazin agar especially might well be utilized for the routine cultivation of these organisms. Even the meningococcus grows in great abundance on this medium, and for a time we were in hopes that it might take the place of serum agar in the case of this organism. We found, however, to our disappointment that while a number of generations may thus be cultivated without any difficulty the organism sooner or later dies out. The observation seemed interesting in principle nevertheless, and seems worthy of further investigation.

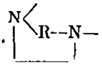
BEHAVIOR OF BASIC DYES. While the rule which was found to hold good in the case of the triphenyl methanes thus seems to apply to all classes of anilin dyes, namely, that the presence of acid auxochromic groups *per se* in predominating number over any basic groups is incompatible with the existence of any inhibitory properties, it does not follow, as we have already seen in the case of the triphenyl and diphenyl methanes, that basicity on the part of a dye of necessity carries with it inhibitory properties. We thus find in the present series that toluidin blue, methylene green, neutral red, anilin yellow, chrysoidin, and vesuvin exhibit no inhibitory power whatever in the concentration which we have employed. On the other hand, it will be seen that the majority of basic dyes possess inhibitory power of a kind, but only in a few is this so extensively developed as in the triaminotriphenyl methanes, and is frequently directed only against a certain group of organisms. Basel blue was thus found to be active only in the case of certain catarrhal micrococci, and to a certain extent also in the case of the Staphylococcus citreus. The same was noted in the case of diazin blue. Methylene violet affected only the anthrax bacillus, actinomyces, a single strain of the catarrhal micrococcus, and the Micrococcus aurantiacus.

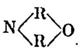
In the case of the triphenyl methanes already it was noted that while some of the dyes were inhibitory for both staphylococci and streptococci, others showed a special selective action for streptococci, while on staphylococci they exercised little or no inhibition. In the groups with which we are dealing in the present paper this was even more marked, and of the entire number of basic dyes only two were markedly inhibitory for both Staphylococcus aureus and streptococci, namely, indazin M and new methylene blue, while echt neutral violet seemed to have a special affinity for staphylococci and azin red, tannin heliotrope, pheno- and tolusafranin, capri blue and coelestin blue, ethylene blue and methylene blue were inhibitory only for streptococci and catarrhal micrococci. As far as the anthrax bacillus and actinomyces are concerned, these were inhibited by practically every dye that had any inhibitory power upon staphylo- or streptococci.

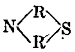
Relation of Chromophoric Group to Inhibition. The fact that so many of the triphenyl methane derivatives are endowed with inhibitory properties strongly suggests that the underlying chromophoric group, which according to Witt is here the complex



(where R represents a benzene radicle) may be responsible for this effect. The thought, hence, suggests itself that in the other inhibitory dyes of basic nature also the chromophore may be operative in this respect. If further investigation should prove this to be the case, we would be forced to the interesting conclusion on the basis of our own observations that of the enormous number of anilin dyes only those can be expected to have inhibitory properties in which the groups







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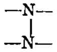

are represented.

Nature of the Inhibitory Action of the Dyes in Question. In considering the nature of the inhibitory action of the dyes in question two possibilities immediately suggest themselves, namely, the existence of a physical as contrasted with a chemical interaction between the dye and the microörganism. As we have shown in our first communication, Stilling inclined to the first possibility, although he felt that chemical influences could not be excluded in the production of the death of the organism. To this view he was without doubt led in great part by the observation that in sufficient concentration the dyes which he had studied would prevent the development of practically all the different bacteria with which he was dealing. But with this phase of the problem we are hardly concerned. On the contrary, we are interested primarily in the fact that in certain dilutions certain dyes will only inhibit the growth of certain organisms. This fact was first pointed out by Rozsahegyi, and was then made the subject of an extended investigation, in reference to one of the most active dyes in this respect, namely, gentian violet, by Churchman. As a result of his investigations, he divides bacteria into a violet positive and a violet negative group. We have shown that these terms have not been well chosen, since the violet color of the dye has really nothing to do with its inhibiting character, but the fact remains that certain organisms are inhibited by certain dyes, while in others there is no evidence of such an inhibition whatsoever (in the concentration 1 to 100,000 chosen by Churchman and myself as standard). This fact in itself, irrespective even of the important bearing of the basicity or acidity of the dye, makes it seem *a priori*, unlikely that the effects should be of a physical character only. But even more significant in this respect is the fact that certain representatives of a susceptible group of organisms which otherwise present the same morphological and cultural characteristics as the remainder may be dye-fast, that is, they are not inhibited by those dyes which interfere with the development of the other members of the same group.

Natural Dye-fast Strains. Churchman already noted an instance of this nature, for he mentions that "in a study of five nearly identical strains belonging to the enteritidis group the selective affinity of the stain (gentian violet) was found to be so specific in its nature as to distinguish between different strains of closely related organisms. On the growth of one member of this group the dye exhibited a constant and complete inhibition; on the other four it was absolutely without any effect."

This differing susceptibility of different members of a given group of organisms to a given dye we could establish also in the case of the staphylococci, the streptococci, and the pneumococci. This is illustrated in Table I, in reference to crystal violet agar (sc., serum agar in the case of the pneumococci), 0 indicating absence of growth, ± faint growth, and +++ abundant growth, as in our first paper.

Table I.—Showing the behavior of different strains of staphylococci, streptococci, and pneumococci in reference to crystal violet (1 in 100,000).

Staphylococcus aureus.

(1)	(40)	(42)	(53)	(58)	(83)	(84)	(95)	(96)
0	0	0	+	0	0	∓	0	0

Staphylococcus Citreus.

(44)
0

Micrococcus Aurantiacus.

(9)
+ to ++

Streptococcus Pyogenes.

(92)	(26)	(28)	(54)	(43)	(50)	(81)	(77)	(61)	(78)	(16)	(37)
++	0	0	++	0	0	0	0	0	0	+++	0

Diplococcus Pneumoniæ.

(102)	(64)	(29)
0	+	0

TABLE II.—Showing the behavior of different strains of staphylococci and streptococci toward various inhibitory dyes.

	Staphylococcus aureus.						Micrococcus catarrhalis.		Streptococcus pyogenes.					
	(1)	(40)	(05)	(42)	(53)	(84)	(100)	(6)	(92)	(85)	(50)	(77)	(16)	(37)
Methyl violet	0	0	0	0	0	0	–	0	0	0	0	+++	+++	0
Crystal violet	0	0	0	0	+	0	–	0	++	0	0	0	+++	0
Hofmann's violet	0	0	0	0	0	±	0	0	++	0	0	+++	+++	0
Benzyl violet	0	0	0	0 to ±	0	0	±	0	0	0	0	0	+++	0
Dahlia	0	±	0	0	0	0	0	0	+++	0	0	+++	+++	0
Fuchsin	0	+++	+++	0	+++	+++	+++	0	+++	0	0	+++	+++	0
New fuchsin	0	0	+++	0	+++	0	0	0	+	0	0	+++	+++	0
Ethyl green	+	+	+ to ++	++	++	0	0	+	0	0	0	0	+++	0
Brilliant green	+++	+	+	0 to ±	+++	0 to ±	0 to ±	++	+	0	0	0	+++	0
Rhodamin 3B	0	0	±	+	+	±	0	+++	++	+++	+++	+++	+++	+++
Rosazein B	0	0 to ±	0	0	0	0	0	0	+ to ++	++	0	0	+++	0
Indazin M	0	+++	0 to ±	0	0	0 to ±	–	0	0	0	0	0	+ to ++	0

This difference in behavior is even more strikingly seen in connection with some of the other inhibitory dyes, as is illustrated in Table II, which likewise shows that even though some of these dyes are closely related, they do not behave in the same manner toward all the strains (see also collective tables).

To explain these individual peculiarities upon a purely physical basis would certainly be difficult, and we are inclined to lay special stress upon this factor as favoring the view that the inhibitory action is of a chemical nature. The likelihood that this may be the case is further emphasized by our observation that it is possible to produce dye-fast strains artificially.

ARTIFICIAL PRODUCTION OF DYE-FAST STRAINS. The observation of Churchman that one single strain only of the B. enteritidis group which he had studied was dye-labile, and our own findings of the occasional deviation from what seems to be the normal for a given group of organisms in reference to a given dye, suggested the idea that this aberration might be viewed as a mutation the outcome of special nutritional conditions, and that it should be possible to produce such mutation artificially. We accordingly planted representatives of the different groups of organisms, which collectively were found to be dye-labile upon agar containing the inhibitory dye, in a dilution of 1 in 10,000,000, and increased this gradually (the rate of progress being determined by the activity of the growth) until the 1 in 100,000 standard was reached. A series of experiments of this order and the ultimate results are shown in Table III.

TABLE III.—Showing the gradual adaptation of different organisms to crystal violet.

Behavior toward	Bacillus subtilis. (4)	Bacillus enteritidis. (8)	Staphylococcus aureus. (42)	Staphylococcus aureus. (1)	Bacillus anthracis. (14)
1 in 100,000 (initial) .	0	±	0	0	0
1 in 10,000,000 . . .	+++	+++	+++	+++	+++
1 in 9,000,000 . . .	+++	+++	+++	+++	+++
1 in 8,000,000 . . .	+++	+++	+++	+++	+++
1 in 7,000,000 . . .	+++	+++	+++	+++	+++
1 in 6,000,000 . . .	+++	+++	+++	+++	+++
1 in 5,000,000 . . .	+++	+++	+++	+++	+++
1 in 4,000,000 . . .	+++	+++	+++	+++	+++
1 in 3,000,000 . . .	+++	+++	+++	+++	+++
1 in 2,000,000 . . .	+++	+++	+++	+++	+++
1 in 1,000,000 . . .	+++	+++	+++	+++	+++
1 in 900,000 . . .	+++	+++	++	+++	+++
1 in 800,000 . . .	+++	+++	++	++	+++
1 in 700,000 . . .	+++	+++	++	++	+++
1 in 600,000 . . .	+++	+++	++	++	+++
1 in 500,000 . . .	+++	+++	++	++	++
1 in 400,000 . . .	+++	+++	+	++	++
1 in 300,000 . . .	++	+++	+	+	+
1 in 200,000 . . .	++	+++	+	+	+
1 in 100,000 . . .	++	+++	+	+	±

While these experiments unquestionably demonstrate the possibility of producing dye-fast strains artificially, it must be admitted that the extent to which this is possible is extremely variable. In the case of the subtilis and the enteritidis strain (of the table) it was merely a matter of transplanting daily to the next strength of agar, while with the other three organisms it was sometimes necessary on entering the higher concentrations to allow growth to continue for a few days in the same concentration before advancing to the next. As the end-result shows the degree of adaptation was also quite variable, and in some instances the 1 in 100,000 mark could not be reached.

In this connection it may be interesting to point out that the B. enteritidis (8) strain which during the greater part of the winter had been dye-labile ultimately became dye-stabile, that is it finally grew quite readily in the presence of all those dyes which in the early fall had inhibited its growth. We cannot help but feel that this change must be viewed as a reversion to what, for that group of organisms, seems to be the normal state. Similarly, we have noted that one streptococcus (16) which originally grew most luxuriantly in the presence of the common inhibitory dyes, is now inhibited by some members of this group (Table IV).

TABLE IV.—Showing reversion of two bacterial strains.

	Original behavior.			Present behavior.		
	Methyl violet.	Dahlia.	Brilliant green.	Methyl violet.	Dahlia.	Brilliant green.
Bacillus enteritidis (8)	0	0	–	++	+++	–
Streptococcus (16)	+++	+++	+++	0	0	0

Upon thinking over the possibilities which have led to this altered behavior we are inclined to refer the change to the fact that whereas during the fall and early winter months the stock cultures of these organisms had been grown on plain agar, they had later been cultivated on beef-juice agar. That apparently insignificant changes in the nutritional surroundings of an organism may actually lead to marked changes in the response of the organism to nutritional stimuli is also shown by an incidental observation which we made when a certain strain of the catarrhal micrococcus had been grown for a comparatively brief period of time on corallin agar and was then transplanted to plain agar and this culture compared with the original (which had not been growing in the presence of corallin). The results are seen in Table V.

TABLE V.—Showing mutation of a catarrhal micrococcus strain under the influence of corallin.

	Methyl violet.	Crystal violet.	Hofmann's violet.
Behavior of original culture (75)	0	0	0
Behavior of culture after having been subjected to influence of corallin (75c)	++	++	+++

If now we attempt an explanation of these various phenomena, namely, the inhibitory action of certain dyes, the existence of natural dye-fast strains, and the possibility of the artificial production of dye fastness, we must bear in mind the fundamental fact that only basic dyes may exhibit inhibitory properties. The most plausible inference, hence, would be to assume the existence of corresponding acid groups in the structure of the organisms in question, with which these basic groups would tend to unite, possibly in a manner corresponding to our concept of the structure of peptids. We could then conceive that the microörganism having anchored the dye in this manner now finds itself unable to bring about the destruction of the molecule with the consequent liberation of that portion which it could utilize in its own metabolism, and the restitution of its own combining receptors. We would thus explain the phenomenon of dye susceptibility upon the same basis upon which Ehrlich explained the action of the toxin molecule upon a cell provided with corresponding receptors, without the necessity, however, of assuming a directly toxic influence. In other words, the cell dies, not necessarily because it has been poisoned, but because a sufficient number of its nutriceptors have been thrown out of action to bring about its starvation, or inability to multiply, as the case may be. This hypothesis would also explain why an acid dye of otherwise the same structure as the corresponding basic inhibitory dye, even though the same number of amino groups should be present, does not interfere with the growth of the organism. For we could readily conceive either that the dye might be anchored by both its basic and acid groups, or if the latter only should be present, by these alone, and that the attack upon or through this group would lead to the destruction of the molecule, and the liberation of the corresponding receptors. In this connection it may not be out of place to refer to the recent observations by Berthelot and Bertrand[3] on the behavior of a certain bacillus, which was isolated by them from the feces, and which was shown to be able either to remove the amino group or the carboxyl group from certain amino-acids and peptids, or both at the same time, according to the nature of the nutritive elements which were otherwise placed at its disposal, showing that the successful attack upon a certain group may lead to the destruction of the nutrient molecule, but that this does not follow of necessity. This is quite in accord with the hypothesis set forth above that the presence of the acid auxochromic group may enable the organism to successfully attack the amino group also, while in the presence of the latter alone this may not be possible. Further investigations will, of course, be necessary to establish the validity of the explanation which has been offered, but it affords even now a possible explanation, where otherwise it would be difficult to account for the observed facts.

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[3] A. Berthelot and D. M. Bertrand, Sur quelques propriétés biochimiques du b. aminophilus intestinalis, Compt. rend. acad. sci., 1912, cliv, No. 26.

The question, then, whether or not a given organism will be inhibited in its growth by a certain dye would, according to our hypothesis, depend primarily upon the existence of suitable receptors on the part of the organism, and secondarily upon its ability to bring about the liberation of such receptors after once they have been occupied by the dye. To these factors two additional ones should be added, namely, the existence or non-existence of other receptors by which the organism could carry on its nutrition (sc., reproduction), and the possibility of producing such receptors, while the others are occupied by the dye.

Upon the preponderance of susceptible receptors over others, or the relative proportion of these, as compared with others that might serve the purpose of nutrition, the extent of the inhibitory effect would, of course, depend, and account for the divers results that have been obtained in our investigations and which are illustrated in the various tables.

The fact that certain strains of an otherwise dye-susceptible group exist in nature or may be produced artificially would seem to warrant the assumption that such receptors either occur preformed or may be produced artificially, and is in itself a support of our hypothesis, and perfectly in accord with the observations of Ehrlich on the occurrence or production of arsenic-fast strains of trypanosomes. Hitherto we have known but little of the possible existence or production of drug-fast bacteria, and so far as our knowledge goes, Morgenthal's observations on the production of drug-fast pneumococcus strains in the animal experiment are the only ones besides our own which go to show that the principle involved applies not only to animal but also to vegetable organisms.

Should our interpretation of the phenomena under investigation be ultimately proved to be correct in principle, even though not in detail, the chemical nature of the inhibitory action of the dyes would seem to be established. At present we think that the evidence which we have furnished points in that direction.

SUMMARY. 1. The inhibitory action upon the growth of certain bacteria which has been shown to be common to all the triaminotriphenyl methanes is not an exclusive property of this group of anilin dyes, but manifested also to a greater or less extent by other strongly basic dyes, and is dependent upon the presence of basic auxochromic groups and the absence of corresponding acid groups (sc., a preponderance of the former over the latter), in association with the existence of certain chromophoric radicles.

2. Some members belonging to dye-susceptible groups of organisms exist in nature which are not inhibited by the dyes in question.

3. Dye susceptibility may be overcome to a greater or less extent by adaptation.

4. The inhibitory action of the dyes in question is probably due to the existence of corresponding (nutri?) receptors in the microörganism to which the dye is anchored, and its inability to bring about the destruction of the dye and the consequent liberation of the corresponding receptors.

5. Natural or artificial adaptation is due to the production of different receptors by which the nutrition (sc., reproduction) of the organism can be carried on, and which either possesses no affinity for the dye in question, or through which the organism can bring about its destruction.

Since writing the above the artificial production of dye-fast strains of bacteria has also been announced from Ehrlich's laboratory.[4]

DIAGNOSTIC VALUE OF PERCUTANEOUS TUBERCULIN TEST (MORO).

By Edward B. Krumbhaar, M.D.,

FELLOW IN RESEARCH MEDICINE, UNIVERSITY OF PENNSYLVANIA; PHYSICIAN TO THE MEDICAL DISPENSARY, PENNSYLVANIA HOSPITAL.

AND

John H. Musser, Jr., M.D.,

PHYSICIAN TO THE PHILADELPHIA GENERAL HOSPITAL; PHYSICIAN TO THE DISPENSARY OF THE PRESBYTERIAN HOSPITAL; INSTRUCTOR IN MEDICINE, UNIVERSITY OF PENNSYLVANIA, PHILADELPHIA.

The specificity of the cutaneous tuberculin reactions is well established;[1] in other words, it is generally acknowledged that a positive reaction to a tuberculin test indicates a previous tuberculous infection, with the production of tuberculous antibodies. For most clinicians, however, and this apparently applies especially to tuberculosis specialists, the extreme sensitiveness of the reaction, whereby even unsuspected or long-cured cases react positively, militates against its practical importance in clinical medicine. Believing that practically all adults have been at one time or another infected by the tubercle bacillus sufficiently to give a positive reaction, they consider such a reaction to be of little use after infancy and of no value in adult life. For instance, Lawrason Brown says: "A positive reaction (of the cutaneous test) after seven or eight years is of little value, as tuberculous infection and clinical tuberculosis are vastly different." This sentiment is so widespread

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[4] Ueber Gewöhnung d. Bakterien an Farbstoffe. Shiga klin. Zeitsch. f. Immunitätsforsch., vol. xviii, p. 65.

[1] Lawrason Brown, Amer. Jour. Med. Sci., 1911, cxlii, 469; L. Hamman and S. Wolman, Arch. Int. Med., 1910, vi, 690, et al.